THE GROWTH OF BACTERIOPHAGE AND LYSIS OF THE HOST

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Introduction and Statement of Main Result

Bacteriophage grows in the presence of living susceptible bacteria. In many but not all cases the growth of phage leads finally to a lysis of the bacterial cells, a phenomenon which in dense cultures manifests itself to the naked eye as a clearing of the bacterial culture. The exact nature of the connection between the growth of the phage and the dissolution of the cells has been a subject of controversy since the original discoveries of d'Herelle in 1917.

D'Herelle believed that lysis is the process by which the phage, which has grown within the bacterium, is liberated from the cell and dispersed in solution. Many later authors, notably Burnet, have concurred with him on this point. Last year Ellis and Delbrück (1) published detailed evidence that phage liberation in *B. coli* occurs in sudden bursts and showed that all the evidence was compatible with the assumption that in sensitive strains the bursts of phage liberation occurred only if and when a cell is lysed.

Northrop and Krueger (3–5) on the other hand have developed ideas along a somewhat different line in the course of their extensive research with a strain of *Staphylococcus aureus* and a bacteriophage active against it. Bordet (2) had put forward the conception that phage production followed by lysis is a more or less normal physiological function of the bacteria. In lysogenic strains where visible lysis never occurs it can be put into close analogy with the production of an extra-cellular enzyme. Northrop's and Krueger's work served to substantiate this view also in their case where the phage growth leads finally to the dissolution of the bacteria. In their view lysis of the bacteria is a secondary and incidental activity of the phage.

Krueger and Northrop (3) found first that clearing, if it occurs at all, begins when a certain threshold value in the ratio total phage/bacteria is

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overstepped. A considerable *loss in total phage* parallels the clearing of the culture. If sufficient phage is added so that the ratio phage/bacteria is greater than the threshold value lysis begins almost at once. This experiment was later repeated and confirmed by Northrop (4, 5) with purified concentrates of phage.

Recently Northrop (5) found with a susceptible *megatherium* strain and homologous phage that the bulk of the phage was liberated *before* the culture began to clear. He found further with a lysogenic strain which never showed clearing but produced phage lysing the sensitive strain, that the yield in phage from this lysogenic strain was large compared to the number of bacteria present in the culture.

All these results indicate that in these strains lysis, if it occurs at all, is brought about by a mass attack of the phage on the bacteria *after* the phage have grown and been liberated into solution.

We have now studied in more detail the relation between phage growth and lysis in a new sensitive strain of *B. coli* and homologous phage and have obtained results which may offer a basis for reconciling the two diverging lines of interpretation.

We have found in this strain two entirely different types of lysis, which we designate as "Lysis from within" and "Lysis from without."

Lysis from without is brought about almost instantly by adsorption of phage at a threshold limit, which is equal to the adsorption capacity of that bacterium. No phage are liberated in this case, on the contrary, the adsorbed phage are lost. The phage attack the cell wall in such a way as to permit swelling of the cell, and its deformation into a spherical body.

Lysis from within is brought about by adsorption of *one* (or few) phage particle(s). Under favorable conditions this one phage particle multiplies during a latent period within the bacterium up to a threshold value (which is equal to the adsorption capacity). When the threshold value is reached, and not before, the phage is liberated by a sudden destruction of the protoplasmic membrane, which permits a rapid exudation of the cell contents without deformation of the cell wall.

It would seem that the results of other observers may be explained by postulating that

(*a*) In the case of *Staphylococcus aureus* the observable clearing is caused by lysis from without. Lysis from within either does not exist here and is replaced by continuous phage secretion; or it exists but leads only to a slow equalization of the refractive indices of the cell interior and the *milieu*. The decrease of total phage during lysis is caused by the adsorption of phage in the process of lysis from without.

(*b*) In the case of *B. megatherium* 36, sensitive, (Northrop (5)) the same relations hold.

(*c*) In the case of *B. megatherium* 899, lysogenic, (Northrop (5)), lysis from without does not occur, although the bacteria can adsorb a few phage particles each. Both the phage production capacity and the phage adsorption capacity are far smaller than the corresponding value for the same phage acting on the sensitive strain.

The equation

$$\text{Adsorption capacity} = \text{maximum yield of phage per bacterium}$$

was found to hold true both for bacteria in the phase of rapid growth, and for saturated bacterial cultures that had been aerated for 24 hours and consisted only of very small bacteria.

This equality points to a material connection between the bacterial constituents which can adsorb the phage and the new phage formed when it grows. These bacterial constituents we shall call *b*. It might be assumed that *b*, which the bacterium constantly produces without the help of phage (and in some cases also secretes), is part of the precursor which under favorable conditions is transformed into phage after combination of the bacterium with a phage particle from without. The complex *bP* might be the catalyst which *in the cell* transforms uncombined *b* into phage. The difference between a sensitive strain and a lysogenic strain would consist in this: in the sensitive strain the reaction

$$b \xrightarrow{\text{catalyzed by } bP} \text{phage (in the cell)}$$

would be faster than the production of *b* (in the cell). In the lysogenic strain *b* would be produced faster than it is converted into phage. This permits the bacterium *and* the phage to grow.

The extremely interesting but puzzling observations of Burnet and McKie (6) on lysogenesis of different variants of one strain of *B. enteriditis* Gaertner, and of Burnet and Lush (7) on induction of resistance and lysogenesis by the phage in a strain of *Staphylococcus albus* may perhaps allow further analysis in the light of these speculations.

EXPERIMENTAL

The strains of B. coli and of homologous phage used in this work were obtained from the Pasadena Junior College, through the courtesy of Mr. F. Gardner. They have not been studied before and will be designated as B_2 and P_2, in distinction to the strains B_1 and P_1 used last year by Ellis and Delbrück (1).

Growth curves of B_2 in Difco nutrient broth at 37° (by colony counts) are shown in Fig. 1. The maximum division rate is considerably smaller than that of B_1.

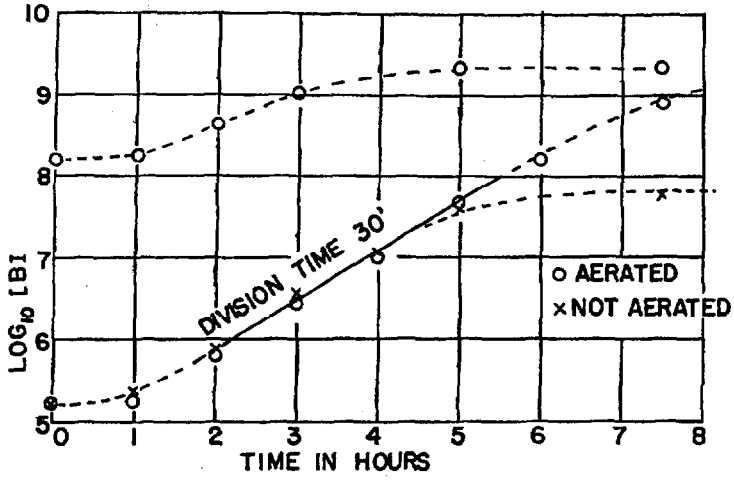

FIG. 1. Growth of bacteria in broth at 37°.

The inoculating bacteria were taken from an 18 hour not aerated broth culture. Without aeration the growth reaches saturation at 10^8 B/cc. With aeration the growth proceeds further beyond 10^9 B/cc. The maximum growth rate is in both cases equal and corresponds to an average division time of 30 minutes. The cessation of growth in the unaerated culture is therefore caused by lack of air. This is further supported by the top curve, which shows how the unaerated 18 hour culture, without the addition of fresh broth, proceeds to grow, when aerated, and reaches about 2×10^9 B/cc.

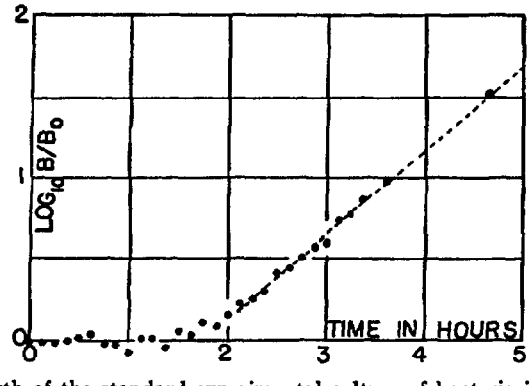

FIG. 2. Growth of the standard experimental culture of bacteria in broth at 37°.

At time zero 0.1 cc. of a 24 hour stock culture was added to 25 cc. broth. Every 7.5 minutes platings were made for colony counts. The plotted values show complete agreement with Hershey's (11) finding that such aerated bacteria exhibit a sudden transition from the phase of cell enlargement (lag period) to the phase of cell division. The lag period is 2 hours, if defined as the time required for increase in cell number by a factor 1.5 (compare Hershey's discussion (11)).

Synthetic Medium.—The bacteria and phage were also grown with aeration in a synthetic medium, consisting of

l-Asparagine	2 gm.
Glucose	4 gm.
Na_2HPO_4 (anhydrous)	6 gm.
KH_2PO_4	3 gm.
$MgSO_4$	0.05 gm.
NaCl	0.05 gm.
Distilled H_2O	1000 cc.

The bacteria in this medium grow more slowly than in broth but attain a higher final concentration. The phage also grow well on bacteria in this medium and cause lysis. But the growth rates of both the bacteria and the phage are only approximately reproducible with different batches of medium. These irregularities must be eliminated before the medium can be used for quantitative studies.

Bacteria transferred from this medium into broth grow at once. Transferred from broth to this medium they require a period of about 24 hours of adaptation before growth begins.

The bacteria were therefore carried on slants of synthetic medium agar and transferred into broth only for the specific experiments.

Stock phage was obtained by lysis in broth and filtration through Jena sintered glass filters. No measurable decrease in titer in periods over 6 months (in contrast to P_1, compare (1)).

Phage assay by plaque count on Difco nutrient agar plates, as described previously (1). The plaques are large (2 mm. diameter) and are countable after 4 hours incubation at 37°.

Turbidity was determined by visual comparison with turbidity standards, in most cases taken from the same culture. Such turbidity determinations are of course very rough, but a refinement of technique in this respect did not seem profitable. The turbidity in any event would not be proportional to the lysis, which in dense cultures, as we have seen, is a complex phenomenon, in which changes in shape, size, and refractive index occur. Each of these factors contributes to the turbidity change.

Preparation of Phage Concentrates.—For the experiments with large excess of phage over bacteria stock phage of very high titer were needed. These were obtained in the following way.

It was observed that lysates obtained from *synthetic medium* cultures (in contrast to broth lysates) lost all their phage on filtration through Jena sintered glass filters. The phage is not inactivated by the filter but simply adsorbed, and it can be eluted with good yields by small volumes of distilled water. An example is given in Table I.

These concentrates could be further concentrated by adding phosphate buffer to restore the original salt concentration and repeating the adsorption-elution procedure.

Filters of the coarser grade 4 were also tried and though effective gave less reliable yields.

Ground glass, silica, and fullers' earth were tried as adsorbents. These also gave good adsorption but the elution was again often unsatisfactory.

The phosphate buffer was replaced by 1 per cent $MgCl_2$ solution and by a 1 per cent

NaCl solution. Both were as effective as phosphates in causing the phage to be adsorbed by the filter.

Finally various concentrations of phosphate buffer were tried in order to determine the lower limit at which adsorption would take place. It was found that reduction of the buffer concentration to one half practically eliminated the adsorption.

By this method several concentrates were obtained with titers between 10^{11}/cc. and 10^{12}/cc. These concentrates were clear in transmitted light and showed intense blue Tyndall scattering. When kept in the ice box they showed no measurable decrease of titer over periods of more than 5 months.

Standard Cultural Conditions.—The cultural conditions of the bacteria were standardized in the following way. A stock culture was kept going for about a week by daily transfers into fresh synthetic medium. At the time the transfer was made, the culture contained about 2.5×10^9 bacteria/cc. These 24 hour aerated bacteria were used for phage assay. They will be referred to as the *stock culture.*

TABLE I

Adsorption and Elution of Phage from Jena Sintered Glass Filters

	Assay	Total phage
25 cc. fresh lysate	1.4×10^{10}	35×10^{10}
Filter lysate through Jena sintered glass filter grade 5 on 3, filtrate	0.001×10^{10}	0.025×10^{10}
Follow with 2 cc. distilled water	6.4×10^{10}	12.8×10^{10}
Follow with 5 cc. distilled water	0.19×10^{10}	0.95×10^{10}

At the time of the transfer of the stock culture a sample of 0.1 cc. was also transferred into 25 cc. *broth* and aerated at 37°. This culture was used for growing the phage, and will be referred to as the *experimental culture.*

Fig. 2 shows the growth of this experimental culture as determined by colony counts in 7.5 minute intervals. It is seen that the bacteria do not start to divide in the first 90 minutes and divide at maximum rate after 3 hours. It can therefore be used to study phage growth either on bacteria that are small and have a long period of growth in size without divisions ahead of them, or on bacteria that have attained their maximum average size and divide at a maximum rate.

Microscopic Observations

We have made some microscopic studies of the lysis of our bacteria under various conditions, both in hanging drop preparations and on nutrient agar plates. The hanging drop preparations have the advantage that one can study changes in mobility of the bacteria and also that the bacteria are subjected to more uniform conditions, while on the agar plate one can follow the history of individual bacteria over a stretch of time. These observations were made at room temperature, the time schedule is therefore retarded in comparison with the growth curves at 37°C.

Great differences in behavior were found depending on whether the bac-

teria were infected with about an equal number of phage or with a large excess of phage (200 to 1 or still higher ratios) and these will be described separately.

1. $P/B = 1$

(*a*) *Hanging Drop*

No changes in the shape of the bacteria were observed, only a gradual diminution in the number of bacteria.

(*b*) *On Nutrient Agar Plates*

The bacteria were first mixed with phage in broth and aerated at 37° for 10 minutes, as in an ordinary growth curve set up. At 10 minutes a 0.1 cc. sample was spread on an agar plate and observed under the microscope (magnification 600). A map was drawn of about 100 bacteria in the field of vision and these were checked every 5 minutes for changes. Up to about 30 minutes (now at room temperature, 25°) only a few bacteria disappeared, the others showed no change. Between 30 and 70 minutes 70 per cent of the bacteria disappeared. They disappeared by a process of fading out, ***without noticeable change in shape.*** The fading out takes about 2 minutes, a faint outline of the rod remaining visible for a long time afterwards. There appeared to be no correlation between the size of the bacterium and the inception of its fading. Many bacteria which at the beginning had a constriction as if they were on the point of dividing behaved as one bacterium on lysis. Sometimes the fading appeared to start at one end of the rod and then to proceed gradually through its entire length.

Discussion.—The bacteria are visible without staining under the microscope by virtue of a difference in the refractive index between their interior and the surrounding medium. The fading out without change of shape means then that the refractive index of its contents becomes equal to that of its ***milieu,*** while the cell wall retains its form. Inside and outside must suddenly become capable of free exchange. This must be due to a sudden disruption of the protoplasmic membrane. A change in permeability could hardly be so drastic as to permit complete equalization in so short a time.

2. $P/B = 200$ or greater

(*a*) *Hanging Drop*

The bacteria kept their normal size and rod shape up to about 20 minutes. Then suddenly within 1 or 2 minutes the large majority was transformed into spherical bodies of about the same volume and small refractive power. These spherical bodies were visible for a long time and only gradually decreased in number, some could be seen much distended and of oval shape.

Besides these spherical bodies there appeared a few very minute rods that were extremely motile.

Also some of the spherical bodies showed great motility.

(*b*) *On Nutrient Agar Plates*

The bacteria were mixed with a 200-fold excess of phage in broth for 5 minutes to permit time for adsorption. Then a 0.1 cc. sample was plated and observation began at 10 minutes. No changes in size or shape were observable up to 18 minutes. Then first few and soon many of the bacteria exhibited a variety of changes in form with parallel slow fading out. In most cases the rod simply swelled to an oval or spherical shape. Sometimes the swelling began at one end giving the impression of a rod attached to a little sphere, gradually the rod shortened and the sphere grew until only the sphere was left, which later assumed an irregular shape and finally faded out. Often the swelling began in the middle at the constriction of a dividing cell

TABLE II

	No. of bacteria lysed by	
P/B	Fading	Swelling
1.3	83	0
1	66	0
1	59	0
25	43	9
150	3	15
250	0	12
300	1	43

and then extended to both ends until only one sphere was visible. The whole process from the inception of swelling to the attainment of a spherical shape took between 2 and 10 minutes. The spheres then fade out very slowly.

Also a few minute rods are seen on the agar plate. They are never lysed. Their genesis has not been observed.

Discussion.—It is clear from the above description that lysis under the influence of many external phage particles is an entirely different phenomenon from lysis under influence of one external phage particle which has grown within the bacterium to a large number. We must distinguish between lysis from within and lysis from without. In the latter case apparently the phage *en masse* attack the cell wall and so alter its elastic properties as to permit swelling of the cell and the uptake of water. Possibly the cell wall is actually dissolved and only the protoplasm remains and swells up freely.

3. P/B between 1 and 200

For these intermediate cases observations on agar plates showed not an intermediate type of lysis but a gradual shift of the *fractions* of bacteria that are lysed by fading or by swelling respectively. Table II illustrates this point.

There is no ambiguity regarding the type of lysis a particular bacterium has undergone, except in rare cases when the fading has proceeded too far by the time of the next inspection, so that the form cannot be ascertained any more.

Bronfenbrenner, Muckenfuss, and Hetler in 1927 (12) and Bayne-Jones and Sandholzer in 1933 (13) have published very interesting photomicrographic moving pictures of lysing bacteria. They describe essentially the same morphological types of lysis which we find. In their experiments, however, the conditions of infection were not systematically varied and the ratio phage/bacteria was in no case determined. The significance of variations in the lytic process was therefore not recognized.

One Step Growth Curves

Phage growth curves with these strains of phage and bacteria show the same general features as those described by Ellis and Delbrück (1) for the strains B_1 and P_1. If phage is added at time zero to an excess of bacteria the plaque count stays constant for 17 minutes, then rises, at first sharply and then more gently (on the logarithmic plot!) until about 30 minutes. At that time the first step is nearly completed. If the growth mixture has not been diluted before the beginning of this first rise another sharp rise begins at about 34 minutes (Fig. 3). If reinfection has been prevented by extreme dilution there is very little further increase in plaque count (see Fig. 2 of the preceding paper).

It was decided to study in more detail this first step. The condition of extreme dilution under which the one step growth curve has to be measured facilitates an accurate analysis, because the samples which have to be plated at definite intervals need only be mixed with bacteria and plated, without further dilution. The time to which the assay is to be referred can then be defined within a fraction of a minute.

Fig. 4 shows the values obtained from three such growth curves. It should be noted that since the plaque count is plotted directly (instead of logarithmically as in most plots of this kind) the sampling error (which is proportional to the measured value) is more conspicuous near the upper end of the growth curve. The actual percentage deviations are experimentally larger near the beginning of the rise, because here the plaque

count increases by a factor of twenty in 3 minutes and very slight inaccuracies in timing will entail huge percentage deviations in the plaque count. During the process of plating a bacterium may liberate the phage which it contains and thus add the full number of a "burst" to the normal sample value. This will place the point too high; it was probably the case in the 17 minute point of the first growth curve.

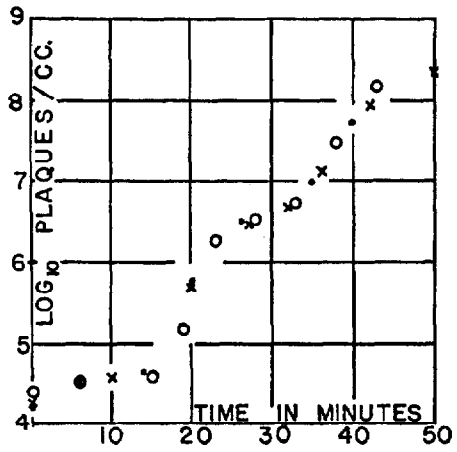

FIG. 3. Phage growth on rapidly dividing bacteria.

At time zero phage were added to the experimental culture of bacteria in its optimal growth phase (3 hours after inoculation) and well below its maximum concentration of bacteria (5×10^7 against 2.5×10^9). The initial phage concentration was 2×10^4, so that even after the first step the bacteria were still much in excess and no multiple infection occurred. After the second step the phage were in excess. The course of events resulting from this situation will be discussed separately.

Dates of experiments: ● 9–13, ○ 9–15, × 9–22.

Fig. 4 brings out very clearly one point which was not recognizable in the logarithmic plots: Phage liberation starts suddenly after the latent period of 17 minutes and continues *at a constant rate* for about 16 minutes, at which point it ceases almost equally abruptly. In this interval from 17 to 33 minutes the plaque count increases by a factor 170.

These characteristics of phage growth, namely the latent period, the spread of the latent period, and the step size depend on the physiological state of the bacteria. For example, if, instead of using the above defined experimental culture after 3 hours when the bacteria are large and divide rapidly we had taken the bacteria from the stock culture directly, the one step phage growth curve under the same conditions (in broth at 37°C.)

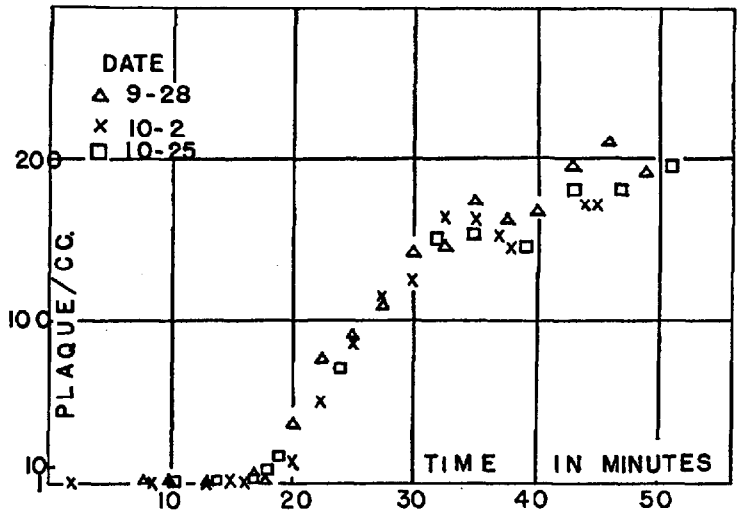

FIG. 4. One step growth curve of phage on rapidly dividing bacteria at 37°. Direct plot.

At time zero about 10^7 phage/cc. were added to 25 cc. of a rapidly growing broth culture of bacteria, that had been aerated for 3 hours and contained about 5×10^7 bacteria/cc. After 5 minutes, 10^3-, 10^4-, and 10^5-fold dilutions of this growth mixture were made in broth of 37° and these were further aerated and incubated. At 1 or 2 minute intervals samples from these mixtures were plated for plaque counts.

It is seen that the plaque count stays constant for 17 minutes, then increases *linearly* with time till 33 minutes when it reaches 170 times the original value. After 33 minutes it stays nearly constant. Phage liberation takes place uniformly during 16 minutes.

It should be noted that on a logarithmic plot the rise would appear to be much more sudden. In fact on such a plot more than half of the step would be accomplished within 3 minutes, when the plaque count has risen to twenty times the original value.

TABLE III

Characteristics of Phage Growth on Rapidly Dividing Bacteria and on 24 Hour Aerated Bacteria, Both Measured in Broth at 37°C., with Aeration

	Minimum latent period	Spread of latent period	Step size	Saturation value
	min.	min.		
Rapidly dividing bacteria	17	16	170	250
24 hrs. aerated bacteria	30	22	20	20

would have been qualitatively the same but quantitatively quite different (see Fig. 5). Table III lists the respective values.

The constant rate of phage liberation in the best one step growth curves

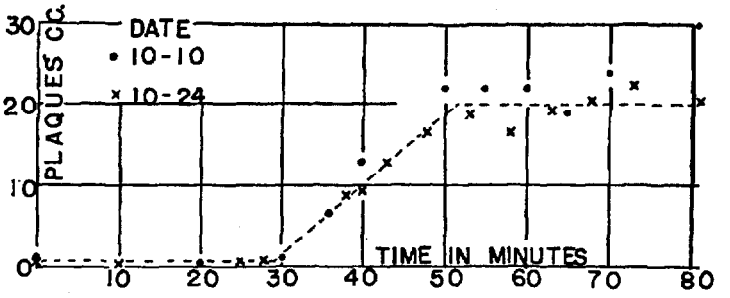

FIG. 5. One step phage growth on 24 hour aerated bacteria. Direct plot.

At time zero 2×10^8 phage/cc. were added to 1 cc. of a 24 hour stock culture of bacteria, containing 2×10^9 bacteria/cc. After 5 minutes free phage were determined and suitable high dilutions in broth were further incubated at 37° with aeration. At intervals samples from these mixtures were plated for plaque count.

It is seen that the plaque count stays constant for 30 minutes, then increases linearly with time till about 50 minutes when it reaches twenty times the original value. After 50 minutes it stays nearly constant. Phage liberation takes place uniformly during 20 minutes.

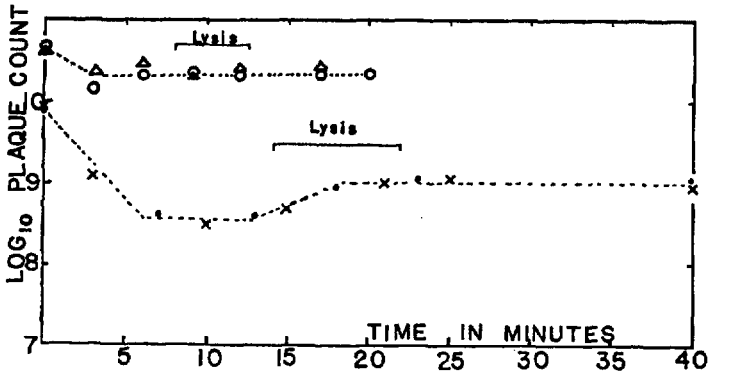

FIG. 6. Addition of a great excess of phage to a growing culture of bacteria, at 37°

	Date	P/B	log P/B	P bound/B	Free phage after adsorption
					per cent
●	9–13	120	2.08	115	4
×	9–22	60	1.78	57	5
○	10–26	500	2.70	250	50
△	11–20	700	2.85	270	60

(Fig. 4) permits a closer analysis. Since the liberation of phage from the *individual bacterium* probably occurs quite suddenly when the bacterium is lysed our result means that the infected bacteria represent a mixture of groups with latent periods ranging between 17 and 33 minutes and that there is a uniform distribution of bacteria over this whole range of latent periods.

The question arises as to what causes a bacterium to have a shorter or longer latent period. Several hypotheses might be suggested, either by ascribing the cause to statistical fluctuations of reactions involving a small number of particles (10), or by connecting it in one way or another with the bacterial cycle. The latter view seems to the author the more likely one but since it has not yet been worked out, further discussion will be deferred.

Multiple Infection

It was reported by Ellis and Delbrück (1) that if a bacterial suspension is infected with an excess of phage no changes occur in the latent period or in the burst size. At that time no phage concentrates were available and the maximum ratio of phage to bacteria attained in that work was only four to one.

We have repeated this work with our new strains and with the concentrates and have been able to work with much higher ratios of phage/bacteria, up to 700 to 1.

Fig. 6 shows some of the results obtained with the high ratios. Samples were assayed every 3 or 5 minutes. Since the assays here require several dilution steps these growth curves are less accurate both with respect to assay values and with respect to timing.

The results show an initial decrease in plaque count because many phage particles are bound to one bacterium which then gives only one plaque. For instance, starting with 10^{10} phage/cc. and a hundred times less bacteria, one finds initially 10^{10} plaques/cc. After 10 minutes only 5 per cent of the phage will be left free; these will give 5×10^8 plaques/cc. In addition the 10^8 bacteria/cc., each having adsorbed on the average 95 phage particles, will give 10^8 plaques/cc., bringing the total plaques to 6×10^8/cc. If the initial ratio phage/bacteria is greater than a certain critical value the bacteria show saturation. This saturation value depends on the physiological state of the bacteria. For instance, for rapidly growing bacteria, if the ratio is 500, the plaque count decreases only by a factor two. The saturation value is therefore 250. On the other hand, for 24 hour aerated bacteria, the saturation value is only about 20. (See Table III, last column.)

We have indicated in the figure the time during which clearing of the mix-

ture occurs. If the initial ratio of phage/bacteria is smaller than the saturation value, clearing occurs only slightly earlier than in a one to one mixture, and it is accompanied by a noticeable increase in the plaque count.

On the other hand if the initial ratio of phage/bacteria is greater than the saturation value, clearing occurs much earlier and is not accompanied by an increase in plaque count.

In both cases the final plaque count is considerably smaller than the initial one; we have, in effect, a phage destruction by the adsorption that causes lysis.

One can see the difference between the two types of lysis with the naked eye. A culture of rod shaped bacteria, like *B. coli*, shows flow lines on shaking due to the orientation of the rods under the influence of the shearing forces of unequal flow. In lyses under the influence of great excess of phage these flow lines disappear before the culture clears up, because the rods are transformed into spherical bodies before they disappear, as described in the section on microscopic observations.

Growth of Phage and Lysis of Bacteria When Equivalent Numbers Are Mixed

It can be predicted that a disturbance must arise when equivalent amounts of phage and bacteria are mixed, due to the fact that the phage that are liberated from the first lysing bacteria will cause an excess of phage over bacteria to be present. These phage will be adsorbed on bacteria that are already infected and will therefore not show up in a plaque count assay. They will moreover interfere with the phage growth in these bacteria and in some of them cause a lysis from without.

Qualitatively the following can be predicted. We have seen that the phage will be liberated at a constant rate (after the lapse of the minimum latent period of 17 minutes). They will be adsorbed at a rate that is proportional to the phage concentration and to the bacterial concentration. The phage concentration is constantly increasing and the bacterial concentration is constantly decreasing (due to lysis). The adsorption rate will therefore pass through a maximum and the net free phage production rate will pass through a minimum. The net result is the appearance of a point of inflection, *i.e.* a secondary step in the phage growth curve, in some cases even a temporary decrease in the free phage if the rate of adsorption at any time exceeds the rate of phage liberation. Because of the loss of phage by adsorption and partial lysis from without the total step size must be smaller than in a one step growth curve where the bacteria are in excess and where multiple adsorption is prevented by extreme dilution, after adsorption of the parent phage.

These predictions are borne out by the experimental results. Fig. 7 shows three such growth curves where nearly equivalent amounts of phage and bacteria were mixed at time zero. The diminished yield is very pronounced and the secondary step is discernible in two sets of observational points. The condition of single infection of all bacteria at zero time can of course be realized only approximately. Even if exactly equivalent amounts

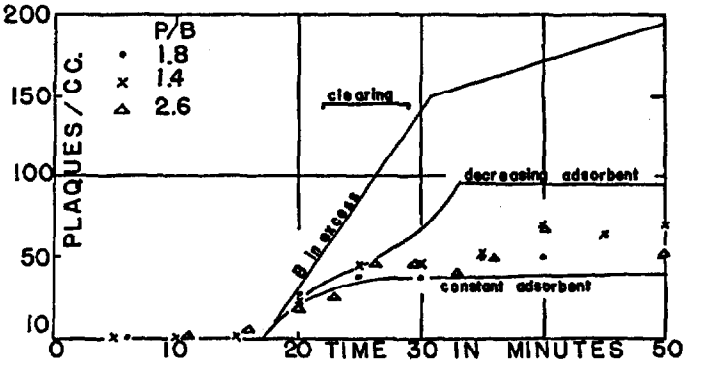

FIG. 7. Growth of phage if equivalent high concentrations ($\sim 10^8$/cc.) of phage and bacteria are mixed at time zero. Direct plot.

Besides the experimental points from three growth curves three theoretical curves are drawn in the figure. These are

1. A one step growth curve with B in excess, taken from Fig. 4.

2. A calculated growth curve, assuming inactivation of the liberated phage on bacteria not yet lysed.

3. Same, but assuming that the adsorbing power of the bacterial constituents responsible for it is unimpaired till the completion of phage liberation and then vanishes abruptly.

The time interval from the beginning of clearing to its completion is indicated. It falls well on the ascending part of the one step growth curve. In the one-to-one growth curves this ascending part is soon counter-balanced by the multiple adsorption loss, so that clearing *seems* to occur during a phase of little phage liberation.

were mixed, the phage would not infect all the bacteria, but distribute themselves according to the probability formulas derived by Poisson. If $P/B = n$ there will be a fraction e^{-n} of the bacteria uninfected. On the other hand our phage assays, though fully reliable as far as relative values go, are not as certain with respect to absolute value, because of the difficulty of obtaining an accurate determination of the efficiency of plating (*cf.* Ellis and Delbrück (1)).

It is not possible to make a complete quantitative prediction of the growth curve because it is not known in detail how the adsorption *en masse* of phage to a bacterium

that is already near a lysis from within will interfere with this process. It is also not quite certain whether those parts of the surface of the bacterium that adsorb the phage will lose their capacity of binding phage immediately upon lysis. In the strains used previously a slow decrease of phage assay after lysis could be ascribed to the continued "adsorption" of phage onto those scattered surface elements. No such decrease of phage assay was ever observed with the new strain. But such observations refer only to inactivation long after lysis and do not tell us whether the adsorbent is instantly destroyed upon lysis.

We have therefore calculated growth curves on the basis of two extreme assumptions.

(*a*) The amount of adsorbent decreases linearly from its initial value to zero during the 16 minutes in which the bacteria are lysed.

(*b*) The amount of adsorbent stays constant at its initial value throughout the course of lysis.

Case (*a*) is described by the differential equation

$$dP/dt = A - kB_0\,(1-t/T)\,P$$

In case (*b*) we have

$$dP/dt = A - kB_0P$$

In these equations the first term, A, represents the phage liberation by lysis during the interval T, as determined in the one step growth curves, the second term is the decrease of phage due to adsorption either on the unlysed bacteria only (case *a*) or on the unlysed bacteria plus the adsorbent from the lysed bacteria (case *b*).

These equations can be integrated explicitly.

We obtain in case (*a*)

$$P = \tfrac{1}{2}A\sqrt{\pi}\,\tau e^{2(T/\tau)^2-(T-t)^2/\tau^2}[G(T/\tau) - G([T-t]/\tau)]$$

with

$$\tau = \sqrt{2T/kB_0}$$

and $G(x)$ the Gaussian integral

$$G(x) = \frac{2}{\sqrt{\pi}}\int_0^x e^{-x^2}\,dx$$

In case (*b*), with constant adsorbent, the adsorption rate grows continuously with the free phage concentration. In this case we have therefore no point of inflection but a continuous asymptotic approach to the final titre

$$P = \frac{A}{kB_0}(1 - e^{-kB_0t}).$$

Since all required constants are known from independent experiments, the particular solutions applying to our case can be evaluated quantitatively. These have been plotted in Fig. 6. The experimental values fall between the limits set by these two cases.

We have also plotted the curve obtained in an ordinary one step growth curve, with B in excess (taken from Fig. 4). The difference between this

curve and the experimental values is the amount of phage lost by adsorption. The data show that about one hundred phage are lost per bacterium. It is clear that this loss depends entirely on the rate of adsorption, which is determined by the product kB_0. If we wish to increase the yield per bacterium we have to decrease either B_0 or k. Reduction of B_0 brings us to the condition employed in the one step growth curves. Here the maximum yield of phage growth per bacterium is obtained, but the actual concentrations of phage are of course very small.

A promising way of increasing the end titre of phage would be to reduce k, the adsorption rate constant. The very interesting experiments of Krueger and his coworkers (8, 9) on the influence of the addition of salts (NaCl and Na_2SO_4) to a growth mixture of phage and bacteria would seem to be completely in accord with the assumption that the adsorption rate constant is diminished in the presence of salt.

In fact, a diminished adsorption rate constant should manifest itself in several ways in a phage growth curve, in which one starts with low concentrations of both phage and bacteria. Namely

1. Delayed clearing, due to delayed adsorption, and therefore delayed phage growth.
2. Higher maximum concentration of bacteria, due to delayed lysis.
3. Higher end titre of phage, due to
(*a*) higher number of bacteria producing phage
(*b*) reduced loss of phage by multiple adsorption.
4. Higher ratio of free to bound phage during the stationary growth phase, due to the fact, that every phage particle spends a longer time in the free state between liberation and adsorption.
5. A period of constant bacterial concentration preceding lysis, when all bacteria are infected and cease to divide but when the phage concentration is not yet sufficient for lysis.

Precisely these five differences from the normal course and no others were noted by Krueger and Strietmann (9) in their study of the influence of the addition of Na_2SO_4.

SUMMARY

1. A new strain of *B. coli* and of phage active against it is described, and the relation between phage growth and lysis has been studied. It has been found that the phage can lyse these bacteria in two distinct ways, which have been designated lysis from within and lysis from without.

2. Lysis from within is caused by infection of a bacterium by a single phage particle and multiplication of this particle up to a threshold value.

The cell contents are then liberated into solution without deformation of the cell wall.

3. Lysis from without is caused by adsorption of phage above a threshold value. The cell contents are liberated by a distension and destruction of the cell wall. The adsorbed phage is not retrieved upon lysis. No new phage is formed.

4. The maximum yield of phage in a lysis from within is equal to the adsorption capacity.

5. Liberation of phage from a culture in which the bacteria have been singly infected proceeds at a constant rate, after the lapse of a minimum latent period, until all the infected bacteria are lysed.

6. If the bacteria are originally not highly in excess, this liberation is soon counterbalanced by multiple adsorption of the liberated phage to bacteria that are already infected. This leads to a reduction of the final yield.

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